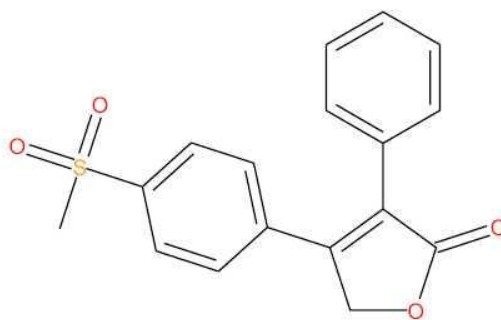




MetaDrug™ Analysis Report

Rofecoxib



Overview of MetaDrug™ Analysis Methodology

MetaDrug combines a suite of chemical structural analysis tools (metabolite prediction, QSAR, structural similarity searching), a comprehensive structure-activity database, and a systems biology database of molecular interactions (protein-protein, compound-protein, protein-enzymatic reaction, compound-enzymatic reaction), canonical signaling and metabolic pathways, and gene-biological property associations (gene-function, gene-disease, gene-toxicity, etc.).

The MetaDrug analysis starts with uploading a chemical structure (Fig. 1). Potential metabolites for the query compound are predicted and separated into major and minor phase 1 and phase 2 metabolites. A suite of pre-defined QSAR models is used to predict possible indications, toxicity issues and ADME properties of the molecule (and, optionally, its metabolites).

MetaDrug uses 3 basic methods to associate compounds to protein targets. **First**, compound's known targets are retrieved from the database (if any of them are described in literature). **Second**, input compound is subjected to similarity search and targets of similar compounds can be ascribed as possible targets of the input molecule. **Third**, QSAR predictions of protein target affinity from the included models or custom derived models define a limited number of potential targets. All 3 methods are available for predicted metabolites if specified in settings.

Possible targets can be then subjected to enrichment analysis to identify pathways and processes that may be affected by the input molecule. Experimental data, for example, expression data associated with compound treatment can be uploaded and overlaid to pathway maps and networks to cross-validate hypothesis about compound's mechanism of action.

To produce this report, MetaDrug was set up to compile list of targets based on known targets of the input molecule and targets of compounds with at least 70% similarity to input molecule. Resulting list was subjected to enrichment analysis across GeneGo Pathway Maps and GO Processes ontologies. No QSAR prediction or metabolite generation were made.

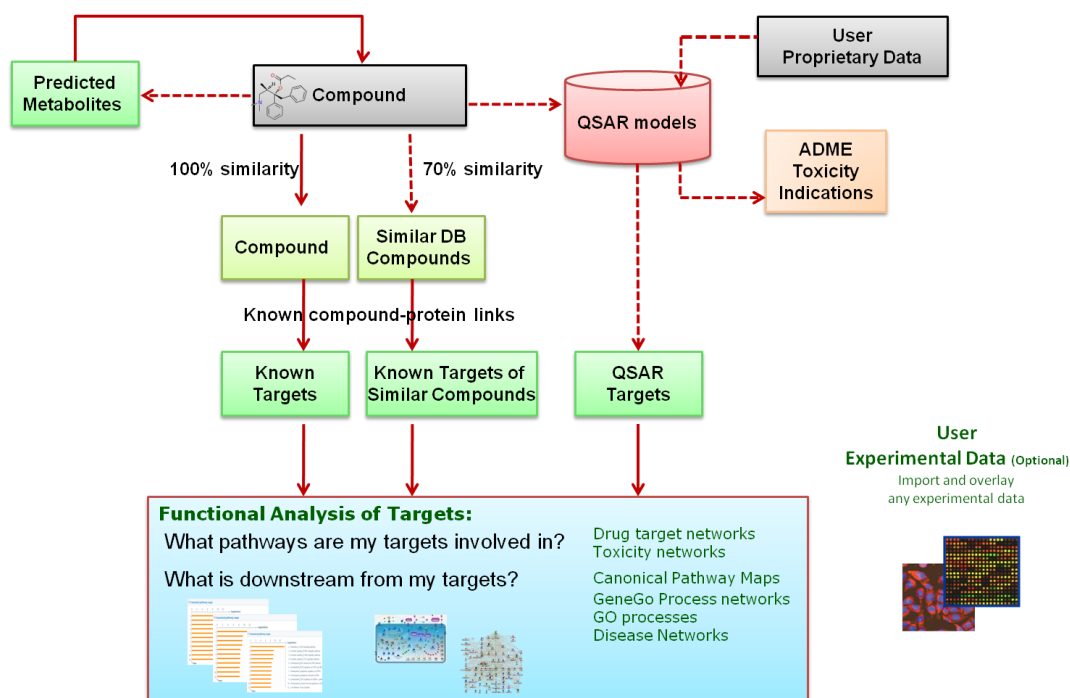


Fig. 1. MetaDrug compound analysis schema.

GeneGo Functional Ontologies

Enrichment analysis consists of matching gene IDs of possible targets with gene IDs in the functional categories representing biological pathways and processes. The probability of a random intersection between a set of IDs of target list with ontology entities is estimated in p -value of hypergeometric intersection. Negative logarithm of p -values serves for category prioritization. The more targets belong to a process/pathway, the lower the p -value, the higher $-\log(p\text{-value})$.

GeneGo Pathway Maps (Fig. 2) ontology represent a set of about 800 signaling and metabolic maps depicting cellular pathways of normal and pathological processes (disease- and toxicity-associated) in a comprehensive way.

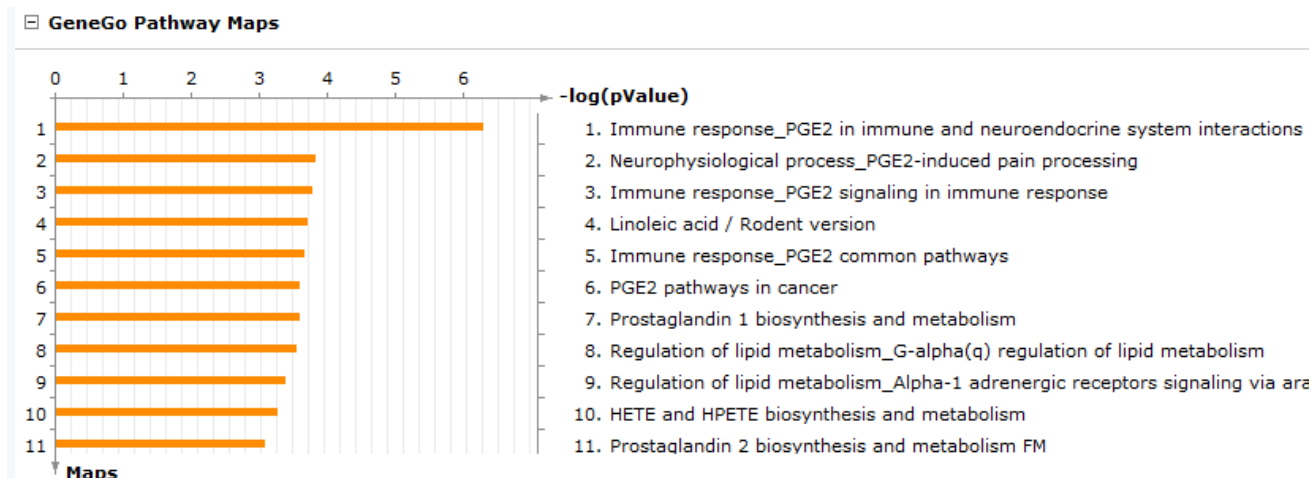


Fig. 2. GeneGo Pathway Maps histogram for studied compound.

GO Processes (Fig. 3) are the original Gene Ontology (GO) cellular processes (<http://www.geneontology.org/>), represented at GeneGo. The processes are structured as hierarchical tree with branches defined according to the Gene Ontology controlled vocabulary.

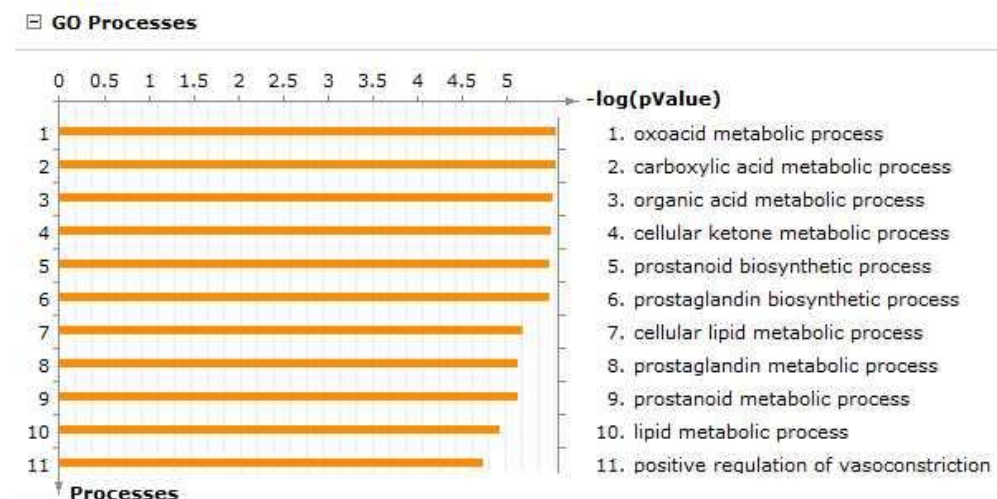


Fig. 3. GO Processes histogram for studied compound.